1006-16 Apoptosis in Human Abdominal Aortic Aneurysms Is Associated With Increased Expression of p53

Angel López-Candales, Dennis R. Holmes, Michael J. Scott, Shixiong Liao, Samuel A. Wickline, Robert W. Thompson. *Washington University School of Medicine, Saint Louis, MO*

Abdominal aortic aneurysms (AAA) are characterized by degeneration of the cellular and matrix components of the aortic wall. We speculate that loss of vascular smooth muscle cells (VSMC) might contribute to medial degeneration and that this might occur through programmed cell death or apoptosis. To determine if apoptosis might play a role in the pathogenesis of AAA, aortic specimens from 5 normal transplant donors and 5 patients undergoing surgical repair of AAA were examined. Using morphometric analysis of α-actin stained tissue sections; the mean density of medial VSMC was reduced by 74% in AAA as compared with normal aorta. Furthermore, ultrastructural analysis of the remaining VSMC demonstrated loss of cell volume and significant alterations in chromatin. *In situ* labeling of fragmented DNA demonstrated extensive nuclear staining, clearly indicative of apoptosis, throughout the medial layers of each aneurysm specimen. No significant areas of positive staining were observed in normal aortas. Because p53 is often associated with the induction of apoptosis, RT-PCR was employed to detect p53 mRNA. A 4-fold increase in p53 expression was observed in AAA specimens as compared to normal aorta. A similar increase in the amount of immunoreactive p53 protein was demonstrated by Western blot and immunohistochemistry. These results indicate that the loss of VSMC that occur with aortic medial degeneration might be due to alterations in p53-mediated cell cycle regulation with the induction of apoptosis and might be responsible in part for the development of aortic aneurysmal disease.

1006-17 Brief Myocardial Ischemic Insult Results in Activation of the Stress Activated Protein Kinase

Surender Kharbanda, Nezam Haider, G. William Dec, Pramod Pandey, Louis Guerrero, Ban-An Khaw, Donald Kufe, Jagat Narula. *Dana-Farber Cancer Institute And Massachusetts General Hospital, Boston, MA*

Acute, severe and prolonged ischemic myocardial insult results in myocardial necrosis. Brief episodes of ischemia do not cause any immediate structural abnormality but their recurrences may subsequently result in cardiomyopathic state and congestive heart failure. Variable amount of myocardial hypertrophy is an obligatory component of cardiomyopathy which results from activation of AP1-binding proteins. We investigated the effect of hypoxia on stress activated protein kinase (SAPK) which is a transactivator of AP1 proteins.

Rat embryonic cardiocytes (H9C2) were subjected to hypoxia for 5, 15, 30, 60, 180, and 360 min. The cells were harvested, lysed and immunoprecipitated with anti-SAPK antibody. In vitro immune complex kinase assays in the protein precipitates using GST-Jun (2–100) fusion protein as a substrate demonstrated an early activation of SAPK activity. There was 10 to 15-fold increase in the SAPK activity at 15–30 min as compared to control cells. The intensity decreased at 60 min and reduced to nearly basal levels by 3 hours.

To investigate pathophysiological significance of SAPK, LAD coronary artery in 5 rats was occluded for 5, 10, 15, 30 and 60 min. The SAPK in the ischemic anterior wall increased to the peak level by 15 min and then reduced significantly to basal levels by 1 H.

Transient transfections of H9C2 cells with wild-type SEK1 and dominant-negative SEK1 vectors (SEK1 K-R) demonstrated SEK1 involvement in SAPK induction. The studies with transient tarnsfections with c-abl and Rac-1/Rho are in progress to elucidate the upstraem cascade activating SEK1.

The immediate activation of SAPK in myocardial cells may explain long-term deleterious effects of brief episodes of hypoxia in evolution of ischemic cardiomyopathy.

1006-18 Can the Geometry of a Coronary Stenosis Predict Occlusion and Myocardial Infarction in the Following Year

François Ledru, Jacques Lespérance, Jean Laurier, Benoit Diebold, Jean-Léon Guermonprez, Pierre Théroux. *Montréal Heart Institute, Montréal (Québec, Canada); Broussais Hospital, Paris (France)*

The risk of myocardial infarction (MI) is known to be poorly related to the severity of pre-existent coronary stenoses. In order to test whether their shape could be a stronger predictive marker, we have studied 38 patients (pts) who underwent a coronary angiogram (angio) less than 12 months (m) before a myocardial infarction (MI) documented by a second angio which allowed for the identification of the culprit lesion and who did not need revascularisation in the interval. The culprit and the other stenoses (control) of the first angio were quantitatively analysed and the following geometric characteristics were measured: percent stenosis, symmetry index (from 0 — totally eccentric — to 1 — perfectly concentric), length, maximal as well as average inflow and outflow angles. The comparison between the 38 culprit and the 130 control stenoses in the same pts gave the following results:

	Culprit	Control	p
Percent stenosis (%)	50.2 ± 13.9	40.1 ± 13.4%	<0.0001
Symmetry index	0.69 ± 0.26	0.49 ± 0.28	<0.0001
Length (mm)	10.3 ± 4.9	8.7 ± 4.8	0.006
Maximal outflow angle (°)	30.3 ± 10.7	23.6 ± 8	<0.0001

The minimal lumen diameter and the other angles were not significant.

In conclusion, the shape of coronary stenoses as defined by the length, the symmetry index and the maximum outflow angle is significantly associated with the subsequent occurrence of a MI, at least in the first year of follow-up.

1006-19 Platelets Are Not Degranulated by a Nonionic Dimeric Contrast Agent During Diagnostic Coronary Angiography and Coronary Interventions

Eric F. Grabowski, Susan E. Boor, Louis J. Rodino, Charles Dow, Robert Holt, Herman Gold, Ik-Kyung Jang, Alan D. Michelson. *Massachusetts General Hospital-Harvard Medical School, Boston MA; University of Massachusetts Medical Center, Worcester MA*

Flow cytometric studies have shown that contrast media (CM) (nonionic iohexol and ionic diatrizoate) degranulate platelets in vitro. To further address this matter, blood from 30 adult patients (pts) undergoing diagnostic cardiac catheterization was added in vitro to equal volumes of ioxaglate (ionic dimer), iodixanol (nonioinic dimer), buffer, or buffer with 10 μM U46619, a thrombin-independent platelet agonist. All pts received IV heparin, had taken ASA 325 mg/day for ≥ 1 day, and were also randomized to receive either ioxaglate or iodixanol. Blood was sampled sequentially from the coronary artery and the right atrium, before and after the administration of CM during diagnostic angiography, and, for a subset of 12 pts, before and during coronary intervention (PTCA and/or stent placement). For the latter pts, heparin was given to assure an ACT of ≥ 275 sec. In vitro, as assayed by whole blood flow cytometry, a small degree of degranulation (as determined by % platelets positive for the α-granule protein P-selectin, mean ± SE) was observed with ioxaglate, but none was seen with iodixanol (Table). In vivo, no degranulation was seen for either CM for any pt probably due to rapid hemodilution of CM with coronary blood flow. Platelet adhesion/aggregation (platelets/cm^2 × 10^6, mean ± SE) to a collagen substrate in whole blood at 270 sec^{-1} was reduced by ioxaglate in vitro, but unaffected by iodixanol in vitro (Table) and by both CM in vivo. No pt suffered a thromboembolic event. In summary, both in vitro and in vivo, the nonionic dimer iodixanol results in neither enhanced platelet adhesion/aggregation under arterial-like rheologic conditions nor platelet degranulation.

	U46619	Buffer	Ioxaglate	Iodixanol
P-selectin	70.5 ± 4.2	3.1 ± 0.3	6.0 ± 0.8	3.0 ± 0.3
Aggregation	N/A	1.7 ± 0.2	0.2 ± 0.04	1.8 ± 0.2

1006-20 The Role of Proteoglycan (Versican) Cleavage by the Metalloenzyme Matrilysin in Unstable Atherosclerotic Lesions in Patients

Igor Halpert, Samuel A. Wickline, Jill D. Roby, Ulrike D. Sires, Thomas N. Wight, Howard G. Welgus, William C. Parks. *Jewish Hospital, Washington University, St. Louis, MO*

Recent descriptions of the expression of matrix metalloproteinases, such as collagenase and gelatinase, in atherosclerotic lesions indicate a potential role in plaque rupture by degrading matrix proteins, which compromises the structural integrity of the lesion. Because components of atherosclerotic lesions also comprise proteoglycans and elastin, we sought to define the sites and cell source of metalloenzymes that could specifically cleave these substances: namely, matrilysin (ML) and macrophage metalloelastase (ME). Samples of lesions from patients undergoing carotid endarterectomy for clinical indications (n = 18) were analyzed by Northern hybridization. Both ML and ME were expressed in atherosclerotic lesions, but not in normal arteries (n = 2). In situ hybridization and immunohistochemistry revealed prominent expression of ML by cells confined strictly to the border between acellular lipid cores and overlying fibrous cap regions. ME was expressed in these same border areas. Staining with CD-68 antibody demonstrated that ML was produced by lipid-laden macrophages, and organ cultures exhibited release of ML from endarterectomy tissue. Immunohistochemical staining for versican demonstrated that this vascular proteoglycan was present at sites of ML expression. Biochemical studies showed that ML degraded versican much more efficiently than did other metalloproteinases present in atherosclerotic lesions. Our findings suggest that the site-specific expression of ML